REMARKS

Claims 1, 2, 4-11, 13, and 14 are pending in this application. Claims 6 and 7 have been canceled, leaving claims 1, 2, 4-5, 8-11, 13, and 14 remaining. Claims 6 and 7 have been canceled in the expectation that the amendments will place this application in condition for allowance. The subject matter of canceled claims 6 and 7 has been incorporated into independent claims 1 and 11.

The amendments do not introduce new matter within the meaning of 35 U.S.C. §132. Basis for the claim amendments is found in claims 1-15 as originally filed; and elsewhere throughout the specification and claims. Accordingly, entry of the amendments is respectfully requested.

Applicants take this opportunity to thank the Examiner for reconsideration and withdrawal of the rejection of claims 1, 2, and 6-11 under 35 U.S.C. §103 as being unpatentable over Kresheck, et al. in view of Pentecost; the rejection of claims 1, 2, and 6-11 under 35 U.S.C. §103 as being unpatentable over Kresheck, et al. in view of Puig; and the rejection of claims 8-10 under 35 U.S.C. §112, second paragraph.

1. Rejection of Claims 1, 2, 4-11, 13, and 14 under 35 U.S.C. §103(a)

The Office Action rejects claims 1, 2, 4-11, 13, and 14 under

35 U.S.C. §103(a), as being unpatentable over Kresheck et al. (US Patent 5,625,053) in view of Pentecost et al. (Eur. J. Biochem. Vol. 195 No. 3 1991) in view of Puig et al. (Biochimica et Biophysica Acta Vol, 1397 No. 1 1998), for the following reasons:

> Kresheck et al. teach a method wherein cells are lysed, an alkaline solution is added, and the DNA is precipitated from the resulting solution with a lower alcohol like methanol. Kresheck et al. also teach the limitations of claims 9 and 10 wherein the RNA is lysed with RNase. See columns 2, 4 and 5. Kresheck et al. fails to teach the method using fish spermatogonium. Kresheck et al. also fails to disclose that the cells were disrupted with a rotating-knife crusher or sonicator.
>
> Puig et al. disclose the acetylation of the lysines in histone, H4, which cause the weakening of the attachment of the histone to the DNA. Applicant's method reads on acetylation since the acylation reaction in the claim is mediated by the anhydride
> compound acetic anhydride.
>
> Pentecost et al. disclose the isolation and extraction of nucleic acids, specifically RNA, from fish spermatogonium. See abstract and page 4873.

Applicants respectfully traverse this rejection. To establish a *prima facie* case, the PTO must satisfy three requirements. First, the prior art reference must teach or suggest all the limitations of the claims. *In re Wilson*, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494, 496 (C.C.P.A. 1970). Second, the prior art relied upon, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated the skilled artisan to modify a reference. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596,

1598 (Fed. Cir. 1988). Lastly, the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1209, 18 U.S.P.Q.2d 1016, 1023 (Fed. Cir. 1991). This rejection fails to establish a prima facie basis for rejection under §103(a), for at least two reasons.

First, the prior art reference must teach or suggest all the limitations of the claims. Contrary to the Office action, Kresheck, et al. does not teach or suggest that an alkaline salt solution, particularly as limited in the claims as amended to a monovalent salt such as sodium nitrate, sodium carbonate, or sodium phosphate, may be used as the lysing agent in a method for purifying genomic DNA; Kresheck, et al. teach the use of alkyldimethylphosphine oxide ("APO"), particularly decyldimethylphosphine oxide (APO-10) and dodecyldimethylphosphine oxide (APO-12) as the *required* active agent for that purpose, with the addition, in a preferred embodiment, of an alkaline salt merely to stabilize the APO. Kresheck, et al. actually teaches away from the inventive subject matter, since it certainly does not teach or suggest the use of an alkaline salt *alone*, as claimed in the present inventive subject matter.

As described in detail in the attached copy of Bhairi, SM,

Detergents: A Guide to the Properties and Uses of Detergents in Biological Systems, CalBiochem Product Booklet, 2001, alkyldimethylphosphine oxides such as APO-10 and APO-12 are non-ionic detergents (see page 35). Detergents are amphipathic molecules that contain both hydrophilic and hydrophobic groups. Because of their amphipathic nature, detergents are able to solubilize hydrophobic compounds in water. Non-ionic detergents contain uncharged hydrophilic head groups and uncharged hydrophobic tails. In general, non-ionic detergents are better suited for breaking lipid-lipid and lipid-protein interactions than protein-protein interactions. Detergents dissolve membranes and solubilize membrane proteins by mimicking the lipid-bilayer environment.

APOs are not readily biodegradable, and cannot be applied as a fertilizer, unlike the end products of the inventive methods. Thus, contrary to the Office Action, whether Kresheck discloses DNA isolation "in the absence of hazardous chemicals such as phenol and chloroform," is not determinative of the unexpected results produced by the inventive methods, which produce isolated DNA (1) "without generating pollutants" and (2) "without resorting to harmful materials such as phenol." It is the absence of environmental pollutants which further distinguishes the inventive subject matter over the prior art of record.

Further, the Examiner has offered no evidence for the mere conclusion that techniques for isolation of plasmid DNA, as shown in Kresheck, et al., are applicable to isolation of genomic DNA. Applicants suggest that it is well known in the art that techniques for isolation of plasmid DNA are generally *inapplicable* to isolation of genomic DNA. Applicants thus respectfully demand that the Examiner either provide a reference to support this startling assertion, or provide a sworn Declaration if she is relying on personal knowledge.

Second, the prior art relied upon, coupled with the knowledge contained in one or more additional prior art references or generally available in the art at the time of the invention, does not contain any suggestion or incentive that would have motivated the skilled artisan to modify a cited reference. Applicants have carefully reviewed all references cited by the Examiner and find no such motivating teaching. The Examiner states that motivation exists in the art, but has cited nothing to support that bare conclusion. Applicants thus respectfully demand that the Examiner either provide a reference to support her conclusion that motivation exists in the art, or provide a sworn Declaration if she is relying on personal knowledge.

The secondary references cited by the Examiner do not remedy these deficiencies. As the Examiner admits, Pentecost, et al.

merely discloses the isolation and extraction of nucleic acids, specifically RNA, from fish spermatogonium. Similarly, Puig, et al. merely discloses the acetylation of the lysines in histone, H4, which cause the weakening of the attachment of the histone to the DNA. Neither Pentecost, et al. nor Puig, et al. discloses the use of sodium nitrate, sodium carbonate, or sodium phosphate as the lysing agent in a method for purifying genomic DNA. Thus, in the absence of any teaching or suggestion in the cited art for using sodium nitrate, sodium carbonate, or sodium phosphate in a method for purifying genomic DNA, with the resulting benefit that the specific salts selected in the inventive subject matter serve the dual purposes of (1) acting as a strong lysis agent and acting as a soil fertilizer upon completion of the claimed methods, and (2) substituting for the detergent(s) generally used in prior art methods, without the resulting environmental contamination of prior art methods. Thus, the claims of the present application are unobvious over Kresheck, et al., Pentecost, et al., and/or Puig et al., either alone or in any combination.

Accordingly, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 1, 2, 4-11, 13, and 14 under 35 U.S.C. §103.

CONCLUSION

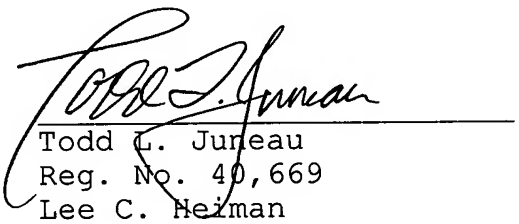
Based upon the above remarks, the presently claimed subject matter is believed to be novel and patentably distinguishable over the prior art of record. The Examiner is therefore respectfully requested to reconsider and withdraw the rejections of claims 1, 2, 4-11, 13, and 14, and allow all pending claims 1, 2, 4-5, 8-11, 13, and 14 presented herein for reconsideration. Favorable action with an early allowance of the claims pending in this application is earnestly solicited.

The Examiner is welcomed to telephone the undersigned attorney if she has any questions or comments.

Respectfully submitted,

NATH & ASSOCIATES PLLC

Date: October 3, 2003

By: 

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CALBIOCHEM®

DETERGENTS

A guide to the properties and uses of detergents in biological systems

By

Srirama M. Bhairi, Ph.D.

A Word To Our Customers

CALBIOCHEM® has been the leading supplier of a variety of quality detergents to a large number of researchers all over the world for almost 50 years. During this period, we have received a number of inquiries on the use of detergents, definitions, relevance of critical micelle concentration (CMC), cloud point, hydrophilic number, and how to select the most appropriate detergent. As a service to the research community, CALBIOCHEM® is providing this guide to the use of detergents in biological systems. The background information and the selected bibliography provided here will hopefully serve the needs of the first time users of detergents as well as those of experienced investigators.

We have also included a chapter on a unique series of compounds known as Non-Detergent Sulfobetaines (NDSBs). As evident from the name, these compounds are not detergents and they do not form micelles. Structurally, NDSBs have hydrophilic groups similar to those found in zwitterionic detergents; however, they possess a much shorter hydrophobic chain. They have been reported to improve the yield of membrane proteins when used in conjunction with the traditional detergents and prevent aggregation during renaturation of chemically or thermally denatured proteins.

The discussion provided in this booklet is by no means complete. However, we hope it will help in the understanding of general principles involved in the use of detergents.

Margaret Dentlinger
Director of Marketing

Table of Contents

Hydrophobic Interactions

Water forms a highly ordered network of intermolecular hydrogen bonds (Figure 1). It is the strength of all the hydrogen bonds combined that imparts the liquid properties to water. Polar or hydrophilic substances dissolve in water because they form hydrogen bonds and electrostatic interactions with water molecules. Non-polar or hydrophobic substances, on the other hand, are unable to form such interactions, and consequently, are immiscible with water. Addition of nonpolar substances to water disrupts intermolecular hydrogen bonding of water molecules and creates a cavity which is devoid of the water molecules. At the surface of the cavity, water molecules rearrange in an orderly manner (Figure 2). This results in a thermodynamically unfavorable decrease in entropy. To compensate for the loss of entropy, water molecules force the hydrophobic molecules to cluster and thus occupy the minimum space. This phenomenon is known as the **hydrophobic effect** and the "forces" between hydrophobic regions are called **hydrophobic interactions**.

Hydrophobic interactions play a major role in defining the native tertiary structure of proteins. Proteins consist of polar and non-polar amino acids. In water-soluble proteins, hydrophobic domains rich in non-polar amino acids are folded in together and thus are shielded from the aqueous environment. In membrane proteins, some hydrophobic regions that otherwise would be exposed to the aqueous environment are surrounded by lipids.

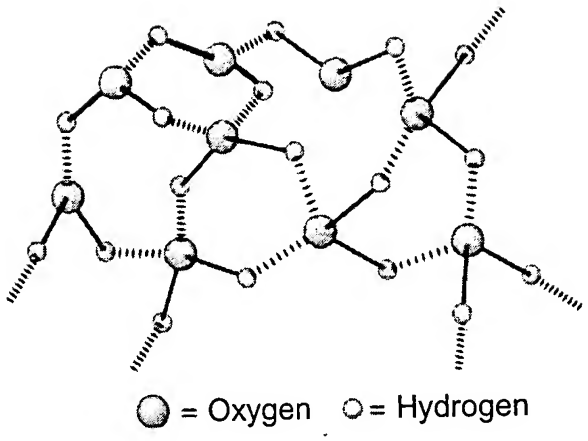

Figure 1: Inter-molecular hydrogen bonding in water.

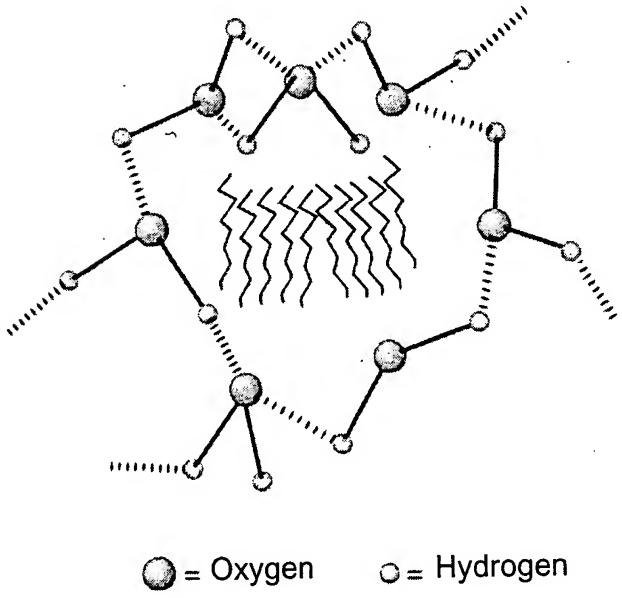

Figure 2: Clustering of hydrocarbon molecules in water.

What are Detergents?

Detergents are amphipathic molecules that contain both polar and hydrophobic groups. These molecules contain a polar group (head) at the end of a long hydrophobic carbon chain (tail). In contrast to purely polar or non-polar molecules, amphipathic molecules exhibit unique properties in water. Their polar group forms hydrogen bonds with water molecules, while the hydrocarbon chains aggregate due to hydrophobic interactions. These properties allow detergents to be soluble in water. In aqueous solutions, they form organized spherical structures called **micelles** (Figure 3), each of which contain several detergent molecules. Because of their amphipathic nature, detergents are able to solubilize hydrophobic compounds in water. Incidentally, one of the methods used to determine the CMC (see below) relies on the ability of detergents to solubilize a hydrophobic dye. Detergents are also known as surfactants because they decrease the surface tension of water.

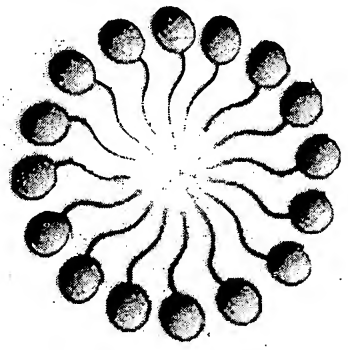

Figure 3: A detergent-micelle in water.

Biological Membranes

Biological membranes are composed of phospholipids and proteins where phospholipids can be viewed as biological detergents. The majority of the lipids that make up the membrane contain two hydrophobic groups connected to a polar head. This molecular architecture allows lipids to form structures called **lipid bilayers**, in which the hydrophobic chains face each other while the polar head groups are outside facing the aqueous milieu (Figure 4). Proteins and lipids, like cholesterol, are embedded in this bilayer. This bilayer model for membranes was first proposed by Singer and Nicolson in 1972 and is known as the **fluid mosaic model** (Figure 5). The embedded proteins are held in the membrane by hydrophobic interactions between the hydrocarbon chains of the lipids and the hydrophobic domains of the proteins. These membrane proteins, known as **integral membrane proteins,** are insoluble in water but are soluble in detergent solutions.

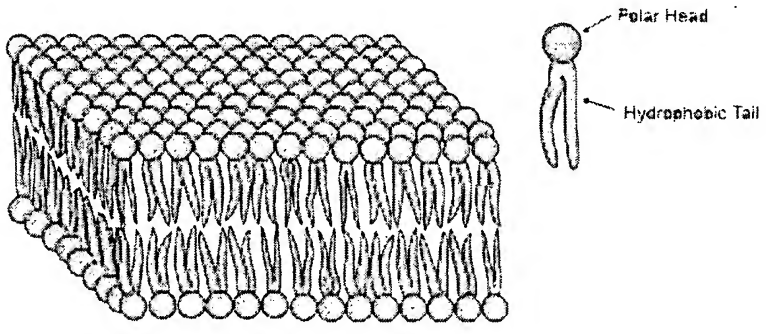

Figure 4: A phospholipid bilayer.

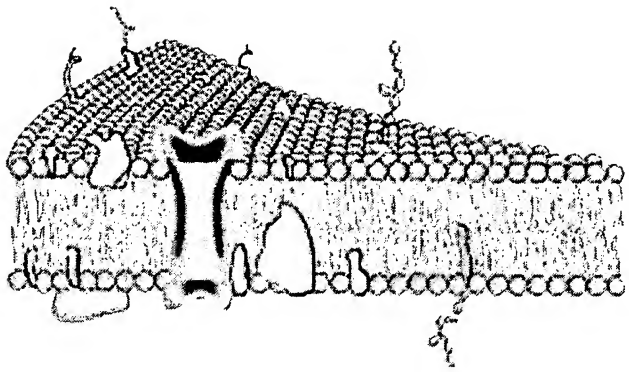

Figure. 5: Fluid-mosaic model of a biological membrane.

How Do Detergents Solubilize Membrane Proteins?

Detergents solubilize membrane proteins by mimicking the lipid-bilayer environment. Micelles formed by detergents are analogous to the bilayers of the biological membranes. Proteins incorporate into these micelles via hydrophobic interactions. Hydrophobic regions of membrane proteins, normally embedded in the membrane lipid bilayer, are now surrounded by a layer of detergent molecules and the hydrophilic portions are exposed to the aqueous medium. This keeps the membrane proteins in solution. Complete removal of detergent could result in aggregation due to the clustering of hydrophobic regions and, hence, may cause precipitation of membrane proteins.

Although, phospholipids can be used as detergents in simulating the bilayer environment, they form large structures, called vesicles, which are not easily amenable for isolation and characterization of membrane proteins. Lyso-phospholipids form micelles that are similar in size to those formed by many detergents. However, they are too expensive to be of general use in everyday protein biochemistry. Hence, the use of synthetic detergents is highly preferred for the isolation of membrane proteins.

Dissolution of membranes by detergents can be divided into different stages (Figure 6). At low concentrations, detergents bind to the membrane by partitioning into the lipid bilayer. At higher concentrations, when the bilayers are saturated with detergents, the membranes disintegrate to form mixed micelles with the detergent molecules. In the detergent-protein mixed micelles, hydrophobic regions of the membrane proteins are surrounded by the hydrophobic chains of micelles. In the final

stages, solubilization of the membranes leads to the formation of mixed micelles consisting of lipids and detergents and detergent micelles containing proteins (usually one protein molecule per micelle). For example, solubilization of a membrane containing rhodopsin by digitonin leads to complexes containing one rhodopsin molecule per micelle consisting of 180 digitonin molecules. Other combinations of micelles containing lipids and detergents and lipid-protein-detergent molecules are possible at intermediate concentrations of detergent. Micelles containing protein-detergent molecules can be separated from other micelles based on their charge, size, or density.

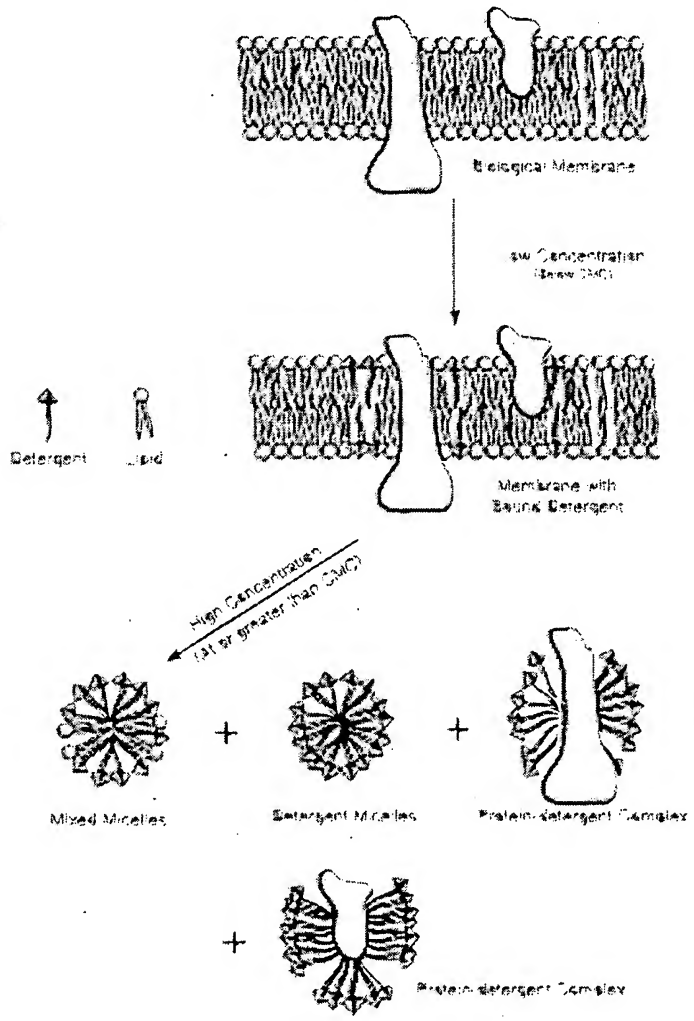

Figure 6: Stages in the dissolution of a biological membrane with detergents.

Classification of Detergents

A large number of detergents with various combinations of hydrophobic and hydrophilic groups are now commercially available. Based on the nature of the hydrophilic head group, they can be broadly classified as **ionic, non-ionic,** and **zwitterionic** detergents.

Ionic Detergents

Ionic detergents contain a head group with a net charge. They can be either negatively (anionic) or positively charged (cationic). For example, sodium dodecyl sulfate (SDS), which contains the negatively charged sulfate group, is an anionic detergent while cetyl trimethyl-ammonium bromide (CTAB), which carries the positively charged trimethylammonium group, is a cationic detergent. Furthermore, the ionic detergents either contain a hydrocarbon (alkyl) straight chain as in SDS and CTAB, or a more complicated rigid steroidal structure as in sodium deoxycholate (see bile acid salts). There is a repulsion between the similarly charged polar groups of detergent molecules in a micelle. Therefore, the size of the micelle is determined by the combined effect of hydrophobic attraction of the side chains and the repulsive forces of the ionic groups. Consequently, neutralizing the charge on the head group with increasing concentrations of a counter ion leads to a larger micellar size. Micellar size also increases with the increase in alkyl chain length.

Bile Acid Salts

Bile acid salts are anionic detergents containing a rigid steroidal hydrophobic group (e.g., sodium salts of cholic acid and deoxycholic acid). In addition to the anionic carboxyl group at the end of the short alkyl chain they also carry hydroxyl groups on the steroid structure. Thus, there is no well-defined polar head group. Instead, the bean shaped molecule has a polar and an apolar face.

Bile acid salts form small aggregates. They can be conjugated to taurine or glycine at the end of the carboxyl group. Unlike spherical micelles formed by alkyl ionic detergents, the micelles formed by bile acid salts are kidney shaped due to their rigid structure. As for ionic detergents, their micellar size is influenced by the concentration of the counter ion. Due to the low pK_a (5 - 6) of the unconjugated bile salt, and low solubility of bile acids, their utility is limited to the alkaline pH range. On the other hand, the pKa of conjugated bile acid salts is much lower, hence, they can be used over a broad pH range. Dihydroxy bile acid salts and deoxycholate are more effective than trihydroxy bile acid

salts in membrane solubilization and in dissociation of protein-protein interactions. Trihydroxy bile acid salts are milder and are better suited for removal by dialysis.

CALBIOCHEM® offers over 20 anionic detergents. Among them you will find BATC (Cat. No. 196950) and TOPPS (*tert*-octyl phenyl propanesulfonic acid) (Cat. No. 615000). BATC (4′-amino-7-benzamido-taurocholic acid), a synthetic derivative of taurocholic acid, is especially suited for solubilization of glycosyl-phosphatidyl inositol (GPI) anchored membrane proteins. TOPPS has the same aromatic hydrophobic tail as TRITON® X-100; however, instead of non-ionic polyoxyethylene groups, it contains an ionic sulfonate group as the hydrophilic head group. This detergent has been used in the renaturation and refolding of chemically and thermally denatured carbonic anhydrase B.

Non-ionic Detergents

Non-ionic detergents contain uncharged, hydrophilic head groups that consist of either polyoxyethylene moieties as in BRIJ® and TRITON® or glycosidic groups as in octyl glucoside and dodecyl maltoside. In general, non-ionic detergents are better suited for breaking lipid-lipid and lipid-protein interactions than protein-protein interactions. Hence, they are considered non-denaturant and are widely used in the isolation of membrane proteins in their biologically active form. Unlike ionic detergents, salts have minimal effect on the micellar size of the non-ionic detergents.

Detergents with polyoxyethylene head groups, may contain alkylpolyethylene ethers with the general formula $C_nH_{2n+1}(OCH_2CH_2)_xOH$, or a phenyl ring between the alkyl chain and the ether group. TRITON® X-100 and NP-40 belong to the latter class (see Table I). Polyoxyethylene chains form random coils and are consequently removed farther from the hydrophobic core of the micelles. Detergents with shorter polyoxyethylene chains form aggregates and viscous solutions in water at room temperature, whereas those with longer chains do not aggregate. It should be noted that detergents containing aromatic rings absorb in the ultraviolet region. They may interfere with spectrophotometric monitoring of proteins at 280 nm. CALBIOCHEM® offers hydrogenated versions of these detergents in which the aromatic rings are reduced and these detergents exhibit relatively low absorption at 280 nm.

Alkyl glycosides have become more popular as nonionic detergents in the isolation of membrane proteins for several reasons. First, they are homogeneous with respect to their composition and structure. Second, several variations of alkyl glycosides containing different combinations of the hydrocarbon chain (cyclic or straight chain) and the polar sugar

group can be easily synthesized in pure forms. Third, subtle differences in the physicochemical properties of alkyl glycosides bearing various alkyl chains, attached to either to a glucose, maltose, or a sucrose head group, can be exploited for selective solubilization of membrane proteins.

Zwitterionic Detergents

Zwitterionic detergents are unique in that they offer the combined properties of ionic and non-ionic detergents. Like non-ionic detergents the zwittergents, including CHAPS and the ZWITTERGENT® 3-X-series, do not possess a net charge, they lack conductivity and electrophoretic mobility, and do not bind to ion-exchange resins. However, like ionic detergents, they are efficient at breaking protein-protein interactions. Zwittergents such as CHAPS are less denaturing than the ZWITTERGENT® 3-X series, possibly owing to their rigid steroid ring structure.

General Properties of Detergents

Critical Micelle Concentration (CMC)

The CMC can be defined as the lowest concentration above which monomers cluster to form micelles. Alternatively, it is the maximum attainable chemical potential (concentration) of the monomer. In reality, micellization occurs over a narrow concentration range rather than at a particular concentration. The CMC decreases with the length of the alkyl chain and increases with the introduction of double bonds and other branched points such as would occur in bile acid salts. Additives, such as urea, that break up water structure also increase the CMC. In ionic detergents, the CMC is reduced by increasing the concentration of counter ions. From a practical point of view, a high CMC is desirable when dialysis is used for the removal of the detergent.

Three of the most popular methods used to determine CMC are surface tension, light scattering, and dye solubilization. Surface tension decreases with the detergent concentration and reaches a minimum around the CMC value. Light scattering as well as the solubility of a hydrophobic dye increase with detergent concentration. The point of inflection on a graph obtained by plotting any of the three parameters vs the detergent concentration corresponds to the CMC of the detergent (Figure 7).

Given the CMC, the concentration of the detergent and the aggregation number (see below), it is possible to calculate the concentration of

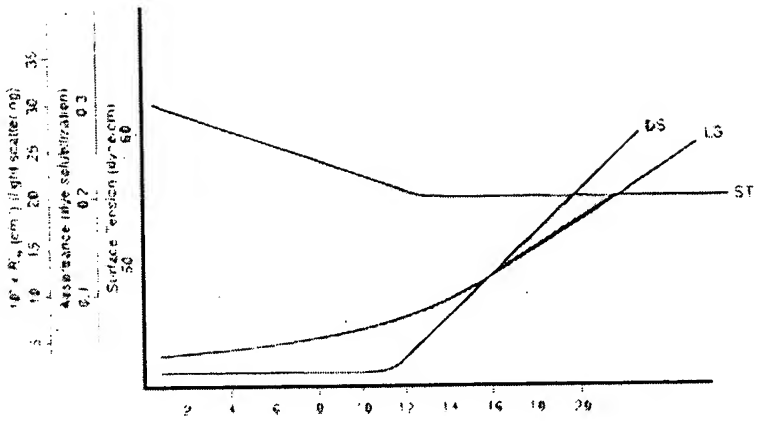

Figure 7: Representative results for determining the CMC of a surfactant by various methods.
Note: DS = dye solubilization; LS = light scattering; ST= surface tension.

micelles in moles per liter using the following formula:

$$[\text{micelles}] = (Cs - CMC) \div N$$

where Cs is the bulk molar concentration of detergent and N is the mean aggregation number. For example, a solution containing 35 mM of CHAPSO (M.W. = 630.9) in PBS buffer will have [(35 - 8) ÷ 11] or 2.45 mM of micelles.

Kraft Point

At very low temperatures, detergents remain mainly in an insoluble crystalline state and are in equilibrium with small amounts of dissolved monomer. As the temperature increases, more and more of the monomeric detergent goes into solution until the concentration of the detergent reaches the CMC. At this point it exists predominantly in the micellar form. The temperature at which the monomer reaches the CMC concentration is called *critical micellar temperature* (CMT). The temperature at which all the three phases - crystalline, micellar and monomeric - exist in equilibrium is called the *Kraft Point* (Figure 8). At this temperature the detergent solution turns clear and the concentration of the detergent reaches its CMC value. For most detergents, the Kraft point is identical to the CMT.

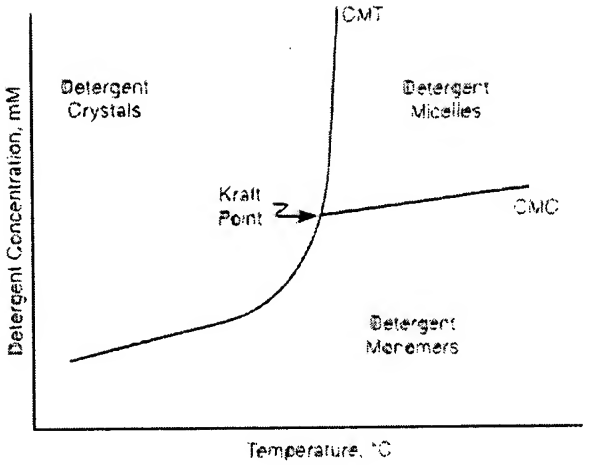

Figure 8: Temperature-composition phase diagram for detergent solutions.

Cloud Point

At a particular temperature above the CMT, non-ionic detergents become cloudy and undergo phase separation to yield a detergent-rich layer and an aqueous layer. This temperature is called the cloud point. Phase separation presumably occurs due to a decrease in hydration of the head group. For example, the *Cloud Point* of TRITON® X-100 is 64°C whereas that for TRITON® X-114 is around 22°C. Hence, TRITON® X-114 solutions are maintained cold. This property can be used to a particular advantage. Membranes can be at first solubilized at 0°C and the solution can be warmed to about 30°C to effect the phase separation. This allows partition of integral membrane proteins into the detergent rich phase which can be later separated by centrifugation.

Aggregation Number

This is the number of monomeric detergent molecules contained in a single micelle. It can be obtained by dividing the micellar molecular weight by the monomeric molecular weight. The molecular weight of micelles can be obtained from various techniques including gel filtration, light scattering, sedimentation equilibrium, and small-angle X-ray scattering. The micelles formed by bile acid salts tend to have low aggregation numbers while those formed by TRITON® have high aggregation numbers. Like micellar size, the aggregation number is also influenced by the ionic strength.

$$\frac{\text{micellar molecular weight}}{\text{monomeric molecular weight}} = \text{aggregation number}$$

Hydrophile-Lipophile Balance (HLB)

This is a measure of hydrophilic character of the detergent: the larger the HLB, the more hydrophilic is the detergent. There appears to be some correlation between the HLB value of a detergent and its ability to solubilize membrane proteins. Detergents with a HLB of 12 to 20 are preferred for non-denaturing solubilization of membrane proteins. Detergents with HLBs in the upper end of the above range are preferred for solubilization of extrinsic proteins. Also, the lower the HLB, the more hydrophobic is the detergent and more easily amenable to removal by hydrophobic chromatography.

Summarizing the above properties, it is evident that the performance of a detergent is dependent on the following factors:

Detergent concentration	Presence of organic additives
Ionic strength	Purity
Length of the alkyl chain	Temperature
pH	

Removal of Unbound Detergents

Excess detergent is normally employed in solubilization of membrane proteins. This is to ensure complete dissolution of the membrane and to provide a large number of micelles such that only one protein molecule is present per micelle. However, for further physicochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent.

Several methods have been used for detergent removal that take advantage of the general properties of detergents: hydrophobicity, CMC, aggregation number and the charge. The following is a brief description of four commonly used methods.

Hydrophobic Adsorption

This method exploits the ability of detergents to bind to hydrophobic resins. For example, CALBIOCHEM®'s CALBIOSORB™ Adsorbent is a hydrophobic, insoluble resin that can be used in batchwise applications to remove excess detergent. Generally, a solution containing a detergent is mixed with a specific amount of the resin and the mixture is allowed to stand at 4°C or room temperature. The resin with the bound detergent can be removed by centrifugation or filtration. For further details, please refer to Appendix 2. This technique is effective for removal of most detergents. If the adsorption of the protein to the resin

is of concern, the resin can be included in a dialysis buffer and the protein dialyzed. However, this usually requires extended dialyzing periods.

Gel Chromatography

Gel chromatography takes advantage of the difference in size between protein-detergent, detergent-lipid, and homogeneous detergent micelles. In most situations protein-detergent micelles elute in the void volume. The elution buffer should contain a detergent below its CMC value to prevent protein aggregation and precipitation.

Separation by gel chromatography is based on size. Hence, parameters that influence micellar size (ionic strength, pH, and temperature) should be kept constant from experiment to experiment to obtain reproducible results.

Dialysis

When detergent solutions are diluted below the CMC, the micelles are dispersed into monomers. The size of the monomers is usually an order of magnitude smaller than that of the micelles and thus can be easily removed by dialysis. If a large dilution is not practical, micelles can be dispersed by other techniques such as the addition of bile acid salts. For detergents with high CMC, dialysis is usually the preferred choice.

Ion-exchange Chromatography

This method exploits the differences in charge between protein-detergent micelles and protein-free detergent micelles. When non-ionic or zwitterionic detergents are used, conditions can be chosen so that the protein-containing micelles are adsorbed on the ion-exchange resin and the protein-free micelles pass through. Adsorbed protein is washed with detergent-free buffer and is eluted by changing either the ionic strength or the pH. Alternatively, the protein can be eluted with an ionic detergent thus replacing the non-ionic detergent.

Guidelines For Choosing a Detergent

A membrane protein is considered solubilized if it is present in the supernatant after one hour centrifugation of a lysate or a homogenate at 100,000 x g. In most cases, it is also important that the biological activity of the protein be preserved in the supernatant after solubilization by a detergent. Hence, the appropriate detergent is that which yields the maximum amount of biologically active protein in the supernatant. Given a large number of detergents available today, choosing an appropriate detergent can be a difficult process. Some of the points outlined below can be helpful in selecting a suitable detergent.

1. The first step is a survey of the literature. A detergent that has been used previously for the isolation and characterization of a protein with similar biochemical or enzymological properties should be tried first.

2. Solubility of the detergent at working temperature can be another consideration. For example, ZWITTERGENT® 3-14 is insoluble in water at 4°C while TRITON® X-114 undergoes a phase separation at room temperature.

3. The method of detergent removal can be of an important consideration. If dialysis is to be employed, a detergent with a high CMC is clearly preferred. Alternatively, if ion exchange chromatography is utilized, a non-ionic detergent or a zwittergent is the detergent of choice.

4. Preservation of biological or enzymological activity may require experimenting with several detergents. Not only the type but also the quantity of the detergent used will affect the activity of the protein. For some proteins biological activity is preserved over a very narrow range of concentration of detergent. Below this range the protein is not solubilized and above a particular concentration, the protein is inactivated.

5. Since TRITON® X-100 contains aromatic rings that absorb at 260-280 nm, this detergent should be avoided if the protocols require UV monitoring of protein concentration. Similarly, ionic detergents should be avoided if the proteins are to be separated by isoelectric focusing. For gel filtration of proteins, detergents with smaller aggregation numbers should be considered.

6. Detergents of utmost purity should be used since some detergents such as TRITON® X-100 are generally known to contain peroxides as contaminants. CALBIOCHEM®'s PROTEIN GRADE® or ULTROL® GRADE detergents that have been purified to minimize these oxidizing contaminants should be preferred.

7. CALBIOCHEM® also offers a variety of Molecular Biology Grade detergents for any research where contaminants such as DNase, RNase, and proteases are crucial.

8. A non-toxic detergent should be preferred over a toxic one. For example, digitonin, a cardiac glycoside, should be handled with special care.

9. For as yet unknown reasons, specific detergents often work better for particular isolation procedures. For example, EMPIGEN BB® (Cat. No. 324690) has been found to be the most efficient detergent in solubilization of keratins while preserving their antigenicity. Similarly, *n*-Dodecyl-β-D-maltoside (Cat. No. 324355) has been found to be the detergent of choice for the isolation of cytochrome c oxidase. Hence, some "trial and error" may be required for determining the most optimum conditions for isolation of a membrane protein in its biologically active form.

10. In some cases, it has been observed that the inclusion of non-detergent sulfobetaines (NDSBs) with detergents in the isolation buffer dramatically improves yields of solubilized membrane proteins.

Appendix 1.

Non-Detergent Sulfobetaines

CALBIOCHEM® is pleased to make available a unique line of products, non-detergent sulfobetaines (NDSBs), for protein chemists. NDSBs are zwitterionic compounds. Like zwittergents, NDSBs carry the sulfobetaine hydrophilic head group. However, in contrast to zwittergents, the hydrophobic group in NDSBs is too short for micellar formation even at concentrations as high as 1 M. Hence, they do not behave like detergents. NDSBs were first employed in native isoelectrofocusing gels to neutralize electrostatic interactions without increasing the conductivity. Recently, they have found use in several applications including isolation of membrane proteins, purification of nuclear and halophilic proteins. Presumably, the contribution from the short hydrophobic groups combined with the charge neutralization ability of the sulfobetaine group result in higher yields of membrane proteins. They have also been used in renaturation and refolding of chemically and thermally denatured proteins. It is hypothesized that the hydrophobic group, although short, interacts with the hydrophobic regions of the protein to prevent aggregation during renaturation. They have been used in renaturation of fusion proteins from inclusion bodies.

NDSBs do not interfere with enzymatic assays involving chromogenic substrates bearing nitrophenyl groups and they do not inhibit the activities of β-galactosidase and alkaline phospatase. In addition, NDSB-195, NDSB-211, and NDSB-221 do not absorb at 280 nm, making them compatible with protein purification procedures in which the protein concentrations are monitored by measuring absorbance at 280 nm.

Product	*Cat. No.	M.W.
NDSB-195	480001	195.3
NDSB-201	480005	201.2
NDSB-211	480013	211.3
NDSB-221	480014	221.3
NDSB-256	480010	257.4

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*You may download product data sheets for these products from our website:
www.calbiochem.com

Appendix 2

CALBIOSORB™ Adsorbent

CALBIOSORB™ Adsorbent (50 ml)
Cat. No. 206550

CALBIOSORB™ Adsorbent, Prepacked Column
(5 ml resin bed + 5 ml buffer reservoir)
Cat. No. 206552

Solubilization of membranes by detergents is essential for their characterization and reconstitution. However, subsequent removal of detergents, particularly the non-ionic detergents with low CMC values, is difficult to achieve.[1,2] Dialysis, the most common method of detergent removal, usually requires about 200-fold excess of detergent-free buffer with three to four changes over several days. However, it is ineffective for removal of detergents of low CMC values. In addition, prolonged exposure to detergents during dialysis can damage certain membrane proteins.[1] Gel filtration, another common method for detergent removal, is highly effective in the reconstitution of AChR,[3] (Ca^{2+} + Mg^{2+})-ATPase,[4] and lactose transporters.[5] However, it gives a broader size distribution of vesicles compared to the dialysis method.[6] Therefore, an expeditious alternative in reconstitution studies is the prior removal of detergents by using a resin capable of effectively binding nondialyzable detergents of low CMC.

CALBIOCHEM® offers an excellent detergent removal product, CALBIOSORB™ Adsorbent. CALBIOSORB™ Adsorbent is a hydrophobic resin that is processed to eliminate unbound organic contaminants, salts, and heavy metal ions and is especially prepared for the removal of detergents from aqueous media. It is supplied in 100 mM Na_2HPO_4, pH 7.0, containing 0.1% sodium azide and can be easily reequilibrated with any other suitable buffer prior to use.

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2. Allen, T., et al. 1980. *Biochim. Biophys. Acta* **601**, 328.
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Table 1. Detergent Adsorption Capacity of CALBIOSORB™ Adsorbent

Detergent	*Mol. Wt.*	*Type*	*Adsorption Capacity (mg detergent/ml resin)*
Cetyltrimethylammonium Bromide (CTAB)	364.5	Cationic	120
CHAPS	614.9	Zwitterionic	110
Cholic Acid, Sodium Salt	430.6	Anionic	73
n-Dodecyl-β-D-maltoside	510.6	Non-ionic	66
n-Hexyl-β-D-glucopyranoside	264.3	Non-ionic	78
Lauryldimethylamine Oxide	229.4	Zwitterionic	66
n-Octyl-β-D-glucopyranoside	292.4	Non-ionic	132
Sodium Dodecyl Sulfate (SDS)	288.5	Anionic	94
n-Tetradecyl-β-D-maltoside	538.6	Non-ionic	161
TRITON® X-100 Detergent	647.0 (Av.)	Non-ionic	157
TWEEN® 20, PROTEIN GRADE® Detergent	1228.0 (Av.)	Non-ionic	122

Detergent absorption capacities were measured by allowing 1.0 g of buffer-free CALBIOSORB™ Adsorbent to equilbrate at room temperature with an excess of detergent (10 ml of 2.0% in H_2O) for 24 hours, then measuring the amount of unabsorbed detergent remaining in the supernatant by gravimetric analysis.

Protocol for Applications Using CALBIOSORB™ Adsorbent, Prepacked Columns

1. Equilibrate the column with 4 to 5 volumes of the sample buffer (e.g., 20 mM sodium phosphate) to remove any sodium azide.
2. Apply the detergent-protein sample to the column.
3. Protein elution from the column may require several column volumes of buffer and can be monitored by UV absorption.

Protocol for Batch Applications Using CALBIOSORB™ Adsorbent

1. Wash CALBIOSORB™ Adsorbent to remove any sodium azide.
2. Calculate the amount of detergent to be removed. For example, 10 ml of 4 mM CHAPS solution contains 24.6 mg of CHAPS.

3. The amount of CALBIOSORB™ Adsorbent required for detergent removal can be determined by inserting the detergent specific adsorption capacity from Table 1 in the following equation:

$$\text{Amount of CALBIOSORB™ Adsorbent} = \frac{\text{Amount of Detergent (mg)}}{\text{Adsorption Capacity (mg/ml)}}$$

(i.e., 24.6 mg CHAPS requires about 0.22 ml of CALBIOSORB™ Adsorbent slurry)

4. Add CALBIOSORB™ Adsorbent directly to the detergent-protein solution. Incubate for 5 minutes at room temperature or for 45 minutes on ice with occasional gentle agitation.

5. Allow the resin to settle. Decant the detergent-free supernatant containing the protein.

6. Dialysis: CALBIOSORB™ Adsorbent may be added directly to a dialysis buffer to facilitate the removal of detergents with low CMC values and to decrease the time required for dialysis when using detergents with higher CMC values. This method is advantageous in that it prevents the adsorption of proteins by the resin.

☞ *A wide variety of application-specific pH and buffer compositions (e.g., HEPES, MOPS, PIPES, Tris, etc.) may be used.*

Storage and Regeneration

Regeneration: Wash with methanol followed by exhaustive washing with water. Reequilibrate with the desired buffer used in the experiment. (NOTE: exhaustive washing is essential to remove methanol from resin). CALBIOSORB™ Adsorbent columns can be used up to ten times before disposal. Regeneration of prepacked CALBIOSORB™ Adsorbent columns is not recommended.

Storage: Wash the resin with a buffer containing 0.1% sodium azide and refrigerate at 4°C.

☞ *When using either the batch or column method, lower ionic strength buffers may decrease the amount of protein absorption by the resin.*

Table 2. Structure and Classification of Detergents

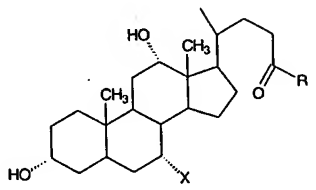
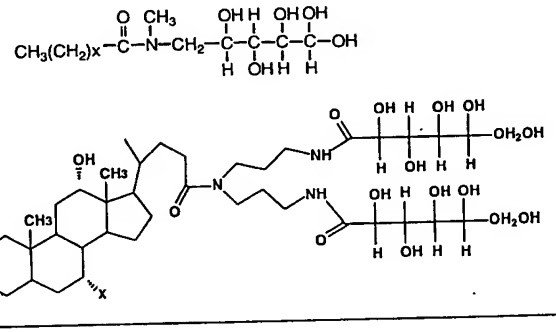
Detergent class	General structure	Examples
Alkyl glycosides	R-O-$(CH_2)_x$-CH_3 R -S-$(CH_2)_x$-CH_3	R = glucose x = 8, *n*-nonyl-β-D-glucopyranoside x = 7, *n*-octyl-β-D-glucopyranoside x = 6, *n*-heptyl-β-D-glucopyranoside x = 5, *n*-hexyl-β-D-glucopyranoside R = maltose x = 11, dodecyl-β-D-maltoside x = 9, decyl-β-D- maltoside R = glucose, x = 7, octyl-β-D-thioglucopyranoside
Bile acids		x = H, R = O-Na^+, sodium deoxycholate x = H, R = $NHCH_2CH_2SO_3$-Na^+, sodium taurodeoxycholate x = H, R = $NHCH_2CO_2$-Na^+, sodium glycodeoxycholate x = OH, R = O-Na+, sodium cholate x = OH, R = $NHCH_2CH_2SO_3$-Na^+, sodium taurocholate x = OH, R = $NHCH_2CO_2$-Na^+, sodium glycocholate
Glucamides		x = 8, MEGA-10 x = 7, MEGA-9 x = 6, MEGA-8 x = H, Deoxy Big CHAP x = OH, Big CHAP

Table 2. Structure and Classification of Detergents (continued)

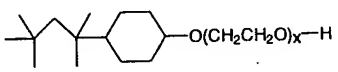
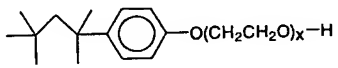
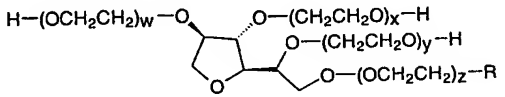
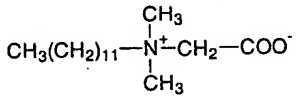
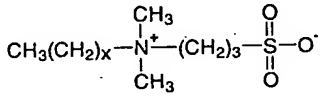
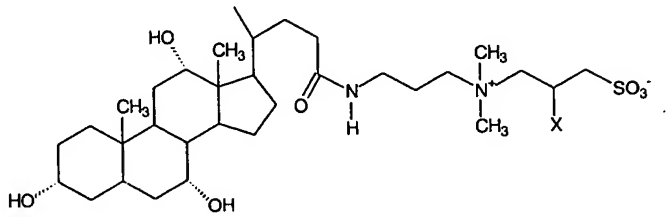
Polyoxyethylenes, monodisperse and polydisperse		x = 9-10, reduced TRITON® X-100 x = 7-8, reduced TRITON® X-114
		x = 9-10, TRITON® X-100, NP-40 x = 7-8, TRITON® X-114
	$CH_3(CH_2)_y$-$O(CH_2CH_2O)_x$-H	y = 12, X = 8, GENAPOL® X-080 y = 12, X = 10, GENAPOL® X-100 y = 11, x = 8, $C_{12}E_8$ y = 11, x = 9, $C_{12}E_9$, THESIT®, LUBROL® PX y = 11, x = 10, GENAPOL® C-100 y = 11, x = 23, BRIJ® 35
	HO(CH_2CH_2O)x-(CH(CH_3)-CH_2O)y-(CH_2CH_2O)-$_z$H	X = 98, Y = 67, Z = 98, PLURONIC® F-127®
	 W + X + Y + Z = 20	R= $C_{11}H_{23}CO_2$- (laurate), TWEEN® 20 R=$C_{17}H_{33}CO_2$- (oleate), TWEEN® 80

Table 2. Structure and Classification of Detergents (continued)

Zwittergents	 pH ≥ 6	EMPIGEN BB® (*n*-dodecyl-N,N-dimethylglycine)
		x = 7, ZWITTERGENT® 3-08 x = 9, ZWITTERGENT® 3-10 x = 11, ZWITTERGENT® 3-12 x = 13, ZWITTERGENT® 3-14 x = 15, ZWITTERGENT® 3-16
		x = H, CHAPS x = OH, CHAPSO

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Non-ionic Detergents

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
APO-10	178375	218.3	4.6	131	28,000	1 g
APO-12	178377	246.4	0.568	2232	500,000	1 g
Big CHAP	200965	878.1	3.4	10	8,800	1 g
Big CHAP, Deoxy	256455	862.1	1.1 - 1.4	8-16	10,500	250 mg 1 g
BRIJ® 35, PROTEIN GRADE® Detergent, 30% Solution	203724	—	0.09	40	48,000	100 ml 1 L
BRIJ® 35, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	203728	—	0.09	40	48,000	50 ml
$C_{12}E_5$	205524	—	—	—	—	1 g
$C_{12}E_6$	205527	450.7	0.087	—	—	1 g
$C_{12}E_8$	205528	538.8	0.11	123	66,000	1 g
$C_{12}E_9$	205529	582.8	0.08	—	—	1 g
Cyclohexyl-*n*-ethyl-β-D-maltoside, ULTROL® Grade	239774	452.5	120	—	—	1 g
Cyclohexyl-*n*-hexyl-β-D-maltoside, ULTROL® Grade	239775	508.6	0.56	63	32,000	500 mg 1 g
Cyclohexyl-*n*-methyl-β-D-maltoside, ULTROL® Grade	239776	438.5	340	—	—	1 g
n-Decanoylsucrose	252721	496.6	2.5	—	—	1 g 5 g
n-Decyl-β-D-maltopyranoside, ULTROL® Grade	252718	482.6	1.6	—	—	1 g 5 g
n-Decyl-β-D-thiomaltoside, ULTROL® Grade	252725	498.6	0.9	—	—	500 mg
Digitonin, High Purity	300410	1229.3	—	5 - 6	7000	250 mg 1 g 5 g

Non-ionic Detergents cont.

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
Digitonin, Alcohol-Soluble, High Purity	300411	1229.3	—	5 - 6	7000	250 mg 1 g
n-Dodecanoylsucrose	324374	524.6	0.3	—	—	1 g 5 g
n-Dodecyl-β-D-glucopyranoside	324351	348.5	0.13	200	70,000	1 g
n-Dodecyl-β-D-maltoside, ULTROL® Grade	324355	510.6	0.1-0.6	98	50,000	500 mg 1 g 5 g 25 g
ELUGENT™ Detergent, 50% Solution	324707	—	—	—	—	100 ml
GENAPOL® C-100, PROTEIN GRADE® Detergent, 10% Solution	345794	627.0	—	—	—	50 ml
GENAPOL® X-80, PROTEIN GRADE® Detergent, 10% Solution	345796	553.0	0.06 - 0.15	—	—	50 ml
GENAPOL® X-100, PROTEIN GRADE® Detergent, 10% Solution	345798	641.0	0.15	88	56,000	50 ml
n-Heptyl-β-D-glucopyranoside	375655	278.3	79	—	—	1 g
n-Heptyl-β-D-thioglucopyranoside, ULTROL® Grade, 10% Solution	375659	294.4	30	—	—	10 ml 50 ml
n-Hexyl-β-D-glucopyranoside	376965	264.3	250	—	—	1 g
MEGA-8, ULTROL® Grade	444926	321.5	58	—	—	1 g 5 g
MEGA-9, ULTROL® Grade	444930	335.5	19 - 25	—	—	1 g 5 g
MEGA-10, ULTROL® Grade	444934	349.5	6 - 7	—	—	1 g 5 g

Non-ionic Detergents cont.

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
n-Nonyl-β-D-glucopyranoside	488285	306.4	6.5	—	—	1 g
NP-40	492015	603.0	0.05-0.3	—	—	100 ml 500 ml 1000 ml
NP-40, PROTEIN GRADE® Detergent, 10% Solution	492017	603.0	0.05-0.3	—	—	50 ml
n-Octanoyl-β-D-glucosylamine (NOGA)	488100	305.4	80	—	—	500 mg 1 g 5 g
n-Octanoylsucrose	494466	468.5	24.4	—	—	1 g 5 g
n-Octyl-β-D-glucopyranoside	494459	292.4	20 - 25	84	25,000	1 g 5 g 25 g
n-Octyl-β-D-glucopyranoside, ULTROL® Grade	494460	292.4	20 - 25	84	25,000	250 mg 1 g 5 g
n-Octyl-β-D-maltopyranoside	494465	454.5	23.4	84	38,000	1 g
n-Octyl-β-D-thioglycopyranoside, ULTROL® Grade	494461	308.4	9	—	—	5 g 25 g
PLURONIC® F-127, PROTEIN GRADE® Detergent, 10% Solution	540025	—	4 - 11	—	—	50 ml
TRITON® X-100	648462	625 (avg.)	0.2 - 0.9	100 - 155	80,000	1 kg
TRITON® X-100, PROTEIN GRADE® Detergent, 10% Solution	648463	625 (avg.)	0.2 - 0.9	100 - 155	80,000	50 ml
TRITON® X-100, Molecular Biology Grade	648466	625 (avg.)	0.2 - 0.9	100 - 155	80,000	50 ml
TRITON® X-100, Hydrogenated	648465	631 (avg.)	0.25	100 - 155	80,000	10 g

Non-ionic Detergents cont.

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
TRITON® X-100, Hydrogenated, PROTEIN GRADE® Detergent, 10% Solution	648464	631 (avg.)	0.25	100 - 155	80,000	10 ml
TRITON® X-114, PROTEIN GRADE® Detergent, 10% Solution	648468	537 (avg.)	0.35	—	—	50 ml
TWEEN® 20	655205	1228 (avg.)	0.059	—	—	250 ml
TWEEN® 20, Molecular Biology Grade	655204	—	0.059	—	—	100 ml
TWEEN® 20, PROTEIN GRADE® Detergent, 10% Solution	655206	1228 (avg.)	0.059	—	—	50 ml
TWEEN® 80, PROTEIN GRADE® Detergent, 10% Solution	655207	1310 (avg.)	0.012	58	76,000	50 ml
n-Undecyl-β-D-maltoside, ULTROL® Grade	662085	496.6	0.59	—	—	500 mg

Ionic Detergents

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
BATC	196950	655.8	—	—	—	500 mg
Cetyltrimethylammonium Bromide (CTAB) Molecular Biology Grade	219374	364.5	1.0	170	62,000	100 g
Chenodeoxycholic Acid, Free Acid	2204	392.6	—	—	—	5 g
Chenodeoxycholic Acid, Sodium Salt	220411	414.6	—	—	—	1 g 5 g
Cholic Acid, Sodium Salt	229101	430.6	9 - 15	2.0	900	50 g 250 g 1 kg
Cholic Acid, Sodium Salt, ULTROL® Grade	229102	430.6	9 - 15	2.0	900	1 g 5 g
Deoxycholic Acid, Sodium Salt	264101	414.6	4 - 8	4-10	1600-4100	25 g 100 g
Deoxycholic Acid, Sodium Salt, ULTROL® Grade	264103	414.6	2 - 6	3-12	1200-4900	5 g 25 g 100 g
7α, 12α-Dihydroxy-5β-cholanic Acid	305705	392.6	—	—	—	100 mg
Glycholic Acid, Sodium Salt	360512	487.6	7.1	2.1	1000	1 g 5 g

Ionic Detergents

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
Glycodeoxycholic Acid, Sodium Salt	361311	471.6	2.1	2.1	900	5 g
Lauroylsarcosine, Sodium Salt	428010	293.4	—	2.0	600	5 g
Sodium *n*-Dodecyl Sulfate (SDS)	428015	288.5	7 - 10	62	18,000	1 kg
Sodium *n*-Dodecyl Sulfate (SDS), High Purity	428016	288.5	7 - 10	62	18,000	25 g
Sodium *n*-Dodecyl Sulfate (SDS), Molecular Biology Grade	428023	288.5	7 - 10	62	18,000	50 g 500 g
Sodium *n*-Dodecyl Sulfate (SDS), 30% Solution	428018	288.5	7 - 10	62	18,000	200 ml
Taurochenodeoxycholic Acid, Sodium Salt	580211	521.7	—	—	—	1 g 5 g
Taurocholic Acid, Sodium Salt	580217	537.7	3 - 11	4	2100	5 g 25 g
Taurocholic Acid, Sodium Salt, ULTROL® Grade	580218	537.7	3 - 11	4	2100	1 g 5 g
Taurodehydrocholic Acid, Sodium Salt	580219	531.6	—	—	—	1 g
Taurodeoxycholic Acid, Sodium Salt	580221	521.7	1 - 4	6	3100	5 g 50 g
Taurolithocholic Acid, Sodium Salt	580352	505.7	—	—	—	1 g 5 g
Tauroursodeoxycholic Acid, Sodium Salt	580549	521.7	—	—	—	1 g 5 g
TOPPS	615000	350.5	4.5	—	—	5 g

Zwitterionic Detergents

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
ASB-14	182750	434.7	—	—	—	5g, 25 g
ASB-16	182755	462.7	—	—	—	5g, 25 g
CHAPS	220201	614.9	6-10	4-14	6000	1 g
						5 g
						10 g
						25 g
CHAPSO	220202	630.9	8	11	7000	1 g
						5 g
DDMAB	252000	299.5	4.3	—	—	5 g
DDMAU	252005	397.7	0.13	—	—	5 g
EMPIGEN BB® Detergent, 30% Solution	324690	272.0	1.6-2.1	—	—	100 ml
Lauryldimethylamine Oxide (LDAO), 30% Solution	428011	229.4	1-2	76	17,000	100 ml
ZWITTERGENT® 3-08 Detergent	693019	279.6	330	—	—	5 g
ZWITTERGENT® 3-10 Detergent	693021	307.6	25-40	41	12,500	5 g
						25 g
ZWITTERGENT® 3-12 Detergent	693015	335.6	2-4	55	18,500	5 g
						25 g
ZWITTERGENT® 3-14 Detergent	693017	363.6	0.1-0.4	83	30,000	5 g
						25 g
						100 g
ZWITTERGENT® 3-16 Detergent	693023	391.6	0.01-0.06	155	60,000	5 g
						25 g

a. Average molecular weights are given for detergents composed of mixtures of chain lengths; b. Temperature: 20 - 25°C

TRADEMARKS